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Influence of temperature on the progamic phase in Citrus

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ABSTRACT

Temperature in the progamic phase is critical for the success of plant sexual reproduction, and new knowledge is needed to optimise breeding programmes to obtain new varieties that adapt to a climate change scenario. Using three male donors and one female recipient in the genus *Citrus*, we evaluated the effect of four temperature regimes on each process in the progamic phase. An innovative method based on microscopic observations of cross sections from pollinated pistils collected daily allowed a comprehensive analysis of pollen tube growth (dynamics and kinetics) along the pistil. Pollen grain germination and stigmatic receptivity were evaluated directly on the stigma, which offers more accurate information than previously reported *in vitro* experiments. Our results showed that warm temperatures reduce the time needed by pollen tubes to reach ovules and accelerate pistil degeneration, while cold temperatures produce the opposite effects. Interestingly, we observed both pollen grain germination and pollen tube growth at 10 °C, which have not been observed in previous studies in citrus. At this temperature, the differences observed in both pollen grain germination and pollen tube growth for different genotypes reflect the adaptation of their sporophytic generation to low temperatures which would enable gametophytic screening to be used as a tool to select better adapted genotypes to different temperature conditions. The differences observed in the growth rates between pollen tubes in each genotype-temperature combination provide an opportunity to explore additional gametophytic selection in this reproductive phase. The capacity to respond to temperature changes in the progamic phase to ensure mating can be useful for breeding programs that focus on obtaining better adapted populations to different temperature conditions.

1. Introduction

Temperature is one of the main environmental conditions that influence the success of plant sexual reproduction (Iizumi et al., 2017; Zhao et al., 2017). Several studies report on the impact of temperature on gametophytic generation and the progamic phase. The effect of high temperatures can be observed on both female and male gametes. However, most previous studies have focused on the temperature effect on the morphology, chemical composition, and functionality of pollen grains (Aloni et al., 2001; Distefano et al., 2018; Koti et al., 2005; Lora et al., 2009; Prasad et al., 2002; Sato et al., 2002). Other studies report on sporophytic generation from the postzygotic stage to the reproductive phase (Hedhly, 2011; Hedhly et al., 2009; Sage et al., 2015; Zinn et al., 2010).

The progamic phase, which elapses from pollination to fertilisation, is one of the most critical phases among the events that take place during the sexual reproduction process in plants. It is a period in which specific interactions between the male gametophyte and the pistil occur. This phase is crucial to achieve successful mating and is extremely vulnerable to prevailing environmental conditions. Temperature strongly affects each process in the progamic phase; *i.e.* stigmatic receptivity, pollen grain germination, pollen tube growth and ovule degeneration (Hedhly, 2011). These processes influence the effective pollination period (EPP), defined for the first time by Williams (1965) in apple as ovule longevity minus the time between pollination and fertilisation. The EPP determines the number of days on which pollination is able to produce non-parthenocarpic fruits. This period has been analysed in many fruit crops, and temperature appears as a crucial influential factor (Sanzol and Herrero, 2001). In citrus, the influence of genotype on EPP under field conditions has been reported by Mesejo

Abbreviations: EPP, effective pollination period; PTG, pollen tube growth; PGG, pollen grain germination; SAL, style abscission line

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et al. (2007), but there is very little information available about how temperature affects this period.

Between pollination and fertilisation, pollen grains germinate on the receptive stigma surface and grow through the pistil to reach a viable ovule. In addition, male-female compatibility is needed for fertilisation to occur. In the case of many citrus genotypes, a gametophytic self-incompatibility system is common, which arrests pollen tube development in the style (Soost, 1965). Therefore, a compatible cross is required to complete ovule fertilisation and subsequent seed formation.

By taking advantage of staining techniques to observe pollen tubes inside pistils, early reports evaluated pollen tube growth at increasing temperatures in *Datura stramonium* (Buchholz and Blakeslee, 2006) and *Oenothera organensis* (Lewis, 1942). Since then, the effect of temperature in the progamic phase has been extensively studied in many herbaceous species, (Coast et al., 2016; Elgersma et al., 1989; Kakani et al., 2005; Koti et al., 2005; Liu et al., 2006; Matsumoto et al., 2012; Mckee and Richards, 1998; Shivanna et al., 1991; Snider et al., 2011a, 2011b) as well as woody species and tree crops (Acar and Kakani, 2010; Gao et al., 2014; Hedhly et al., 2005b, 2005a, 2004; Huang et al., 2010; Jefferies et al., 1982; Jefferies and Brain, 1984; Kakani et al., 2002; Koubouris et al., 2009; Luza et al., 1987; Nygaard, 1969; Pham et al., 2015; Radičević et al., 2016; Sedgley, 1977).

Regarding the female counterpart, the evolution of flowers after anthesis until senescence includes basipetal maturation that starts at the stigma and continues downwardly to the ovary. These changes are developmentally regulated and do not, therefore, depend on the action of pollen tubes (Distefano et al., 2011). The pistil senescence process includes loss of stigmatic receptivity, style abscission and ovule degeneration. The influence of temperature on stigmatic receptivity has been reported in woody species, such as sweet cherry (Hedhly et al., 2003), peach (Hedhly et al., 2005a), and cherimoya (Lora et al., 2011), while the influence of temperature on ovule degeneration has been described in plum, and sweet and sour cherry cultivars (Beppu et al., 2001; Cerović et al., 2000; Postweiler et al., 1985).

Citrus (*Citrus* spp.) is the leading fruit crop worldwide, whose production amounts to more than 146 million tons (FAOSTAT, 2017) in more than 100 countries with tropical and subtropical climates (between 40 °N and 40 °S, approx.), and even in colder areas like Japan and the Jeju Island in South Korea. Studies on global climate change predict an increase in average temperatures between 0.3 and 4.8 °C in 80 years (IPCC, 2014), and in the temperature range amplitude, which could limit plant cultivation in some areas. The consequences of the global climate change are already affecting phenological plant traits, especially those related to flowering (Hedhly et al., 2009; Springate and Kover, 2014), and also shifts the expected geographical distribution in natural ecosystems (Corlett and Westcott, 2013; Singer et al., 2016). In this context, the environmental conditions of the main citrus production areas will change and citrus-growing areas may be extended. Thus breeding programs based on sexual hybridisations could take into account new environmental conditions. Very few studies have evaluated the influence of temperature on the progamic phase in citrus, especially those related with the female parent. Distefano et al. (2012) showed that temperature variation in this phase has a strong effect on pollen germination *in vitro* and on the pollen-pistil interaction in detached flowers of three ancestral citrus species. However, new methods based on *in planta* evaluations need to be implemented to characterise the influence of temperature on the progamic phase in citrus, specifically in a more comprehensive and nature-representative way.

Knowledge of how temperature influences the progamic phase in citrus is most important to adapt breeding programmes to a climate change context, and to also establish improved pollination protocols based on controlled environmental conditions. Citrus breeding programmes based on sexual hybridisations have been developed worldwide at both the diploid and triploid levels. At the Instituto Valenciano de Investigaciones Agrarias (IVIA), we have been performing a large-scale triploid breeding programme based on sexual hybridisation since 1996, with more than 16,000 hybrids obtained from more than 300 parental combinations (Navarro et al., 2015). The experience acquired over more than 20 years of hybridisations reveals the importance of temperature in the progamic phase and of the male-female interaction on hybrid production. Indeed major variations between different years and locations have been observed in terms of the number of hybrids recovered from the same hybridisation, which evidences the influence of environmental conditions on pollen and pistil performance (Aleza et al., 2012b, 2012a, 2010).

This paper evaluates the influence of temperature on both male and female parts in the progamic phase of citrus. Experiments were performed *in planta* under three constant temperature regimes, 10 °C, 20 °C and 30 °C, representing cool to hot spring temperatures and the field conditions in the Mediterranean region of Moncada, Valencia, Spain. Alternatively to classic whole pistils staining and squashing protocols, we made histological observations on several cross sections along pistils. This new methodology allowed us to perform a more comprehensive analysis of the dynamics and kinetics of pollen tube growth. The objective of this study was to generate knowledge about the influence of temperature on the progamic phase applicable to future breeding programmes in a climate change context.

2. Material and methods

2.1. Plant material

The influence of temperature on the progamic phase was evaluated using 'Clemenules' clementine (*C. clementina*), 'Pineapple' sweet orange (*C. sinensis* (L.) Osb.) and 'Ichang' papeda (*C. ichangensis* Swing) as the male parents, all crossed with 'Fortune' mandarin (*C. clementina* x *C. tangerina*) as the female parent. 'Clemenules' clementine is the most representative mandarin cultivar grown in Spain. 'Pineapple' sweet orange is a widely used cultivar for juice production, and is reported to be more sensitive to frost than most other varieties. 'Ichang' papeda is a remarkable plant, reported to be the most cold-resistant of all the evergreen species in the citrus group (Hodgson, 1967). The female parent 'Fortune' mandarin is a high quality variety that is widely used as a female parent in breeding programmes due to not only its late maturity and fruit quality, but also because it produces a high frequency of unreduced gametes for triploid hybrid production (Aleza et al., 2010).

2.2. Experimental procedures for pollinations

As our research focused on the progamic phase, experiments were carried out from pollination. The development of sexual organs of both the male and female parents took place herein under the same field conditions (FC), and no differences during gametogenesis due to temperature were assumed between genotypes.

Eight adult 'Fortune' mandarin trees grown in containers under FC were used for the experiments. These plants were six years-old after grafting, that had a canopy between 40–50 cm in diameter and produce terminal flowers that are the adequate to have the highest chance of fruit set (Erner and Shomer, 1996). During the flowering period, six of them were moved by placing two of them in culture chambers in each studied temperature regime: 10(± 2) ºC; 20(± 2) ºC, 30(± 2) ºC. Plants were exposed to 80 μE m^{-2} s^{-1} illumination16 h daily. The remaining two were left under FC. The average temperature under FC within the experimental time frame was 18.5 °C, with a typically gradual increase of up to 30 °C in the daytime and one less than 10 °C at night. The temperature dates were acquired by one automatic weather station located at IVIA (Moncada, Valencia, Spain) (IVIA, 2019). To perform hand-pollinations, anthers were removed from the flowers of the donors randomly harvested at the balloon stage and were dried in Petri dishes over silica gel in a desiccator at room temperature. Dehiscence occurred after one to two days, and the dehiscent anthers were

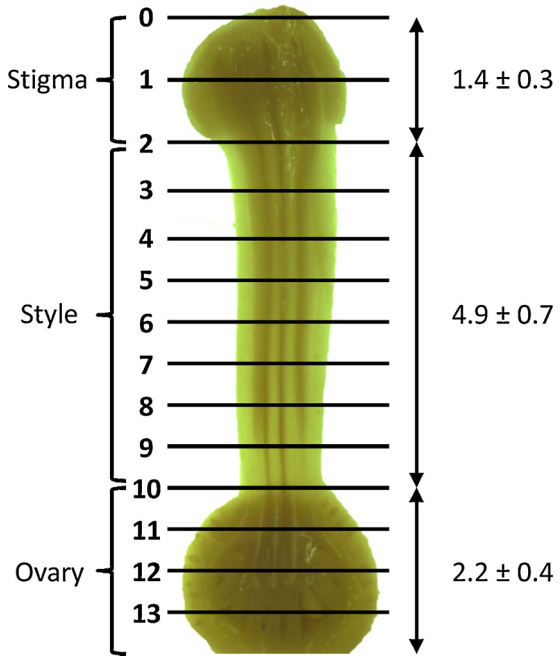

Fig. 1. Pistil longitudinal section. The cross-sections used in this study are indicated with lines from 0 to 13. Length in mm (mean ± SD) of the stigma, style and ovary.

used to pollinate the emasculated flowers at anthesis of the 'Fortune' mandarin plants placed under the different temperature regimes. The pollinated flowers were labelled and bagged to avoid any undesired cross-pollination. The pistils from the pollinated flowers were fixed in FAA solution (formalin, glacial acetic acid, 70% ethanol, 1:1:18, v/v) (Johansen, 1940) and stored at 4 °C until the histological observations. The time that elapsed from pollination to pistil fixation in FAA differed for each experiment, as described below.

2.3. Histological and microscopic observations

In order to evaluate pollen grain germination, pollen tube growth, stigmatic receptivity and ovule degeneration, histological preparations were performed. The pistils fixed in FAA were submerged three times in water for one hour. Pistil length was recorded and they were sliced into 14 cross-sections (0–13) using a sharp blade. Stigmas were sliced into two sections (0–2), styles into eight sections (2–10) and ovaries into four sections (10–13) (Fig. 1). Slices were then stained with 0.1% aniline blue in 0.1 N K_3PO_4 (Linskens and Esser, 1957) and preparations were observed under a Leica MZ16FA stereomicroscope equipped with GFP1 epifluorescence.

As slicing is an innovative method for pollen tube growth characterisations, we compared slicing and the classical squashing approach in our initial research process stages to ensure that observations were consistent. Having verified this methodological consistency, pollen tube growth observations based on slicing were made.

2.4. Evaluation of pollen grain germination (PGG) in planta

To evaluate the effect of temperature and genotype on PGG *in planta*, five flowers of the 'Fortune' mandarin trees placed at 10 °C, 20 °C and 30 °C (FC were not used for the PGG evaluation) were hand-pollinated with pollen from the three male parents. The pollinated flowers were sampled 12–24 h after pollination. PGG was quantified directly on the stigma surface where pollen germination took place. Then the stigma surface was squashed to count the germination of pollen grains (Fig. 2). Pollen grains were scored as germinated when pollen tube length exceeded the diameter of its pollen grain. Five stigmas for each genotype-temperature combination were used and at least 500 pollen grains per stigma were counted with the ImageJ2 software (Schindelin et al., 2015).

2.5. Evaluation of pollen tube growth (PTG) in planta

Five pollinated pistils of each cross and temperature regime (10 °C, 20 °C, 30 °C and FC) were sampled sequentially on ten consecutive days, starting on the day after pollination. The histological observations of the fixed pistils were made to track PTG *in planta* by scoring the number of pollen tubes observed in each pistil section. PTG dynamics was determined by the five maximum values for the number of pollen tubes observed in each pistil section during the ten-day sampling period. PTG kinetics was determined by the pistil section reached by pollen tubes daily. Ovary sections were excluded from the PTG analysis.

2.6. Stigmatic receptivity

Fifty flowers of the 'Fortune' mandarin cultivated at 10 °C, 20 °C, 30 °C and FC were emasculated and labelled at the balloon stage. Five of the labelled flowers for each temperature regime were pollinated sequentially on ten consecutive days, starting on the day of anthesis. 'Ichang' papeda was used as the pollen donor for this experiment. On day one after pollination, pistils were fixed in FAA solution and stored at 4 °C until the histological observations. For each sample, the percentage of the germinated pollen grains and the growth capacity of pollen tubes were evaluated. The percentage of the germinated pollen grains was scored by squashing the stigma surface as described before for PGG (Fig. 2). The growth capacity of pollen tubes was evaluated by counting the number of pollen tubes growing in the middle section of the stigma (Section 1; Fig. 1). A comparison between the samples pollinated on different numbers of days after anthesis in each temperature regime was made to evaluate changes in stigmatic receptivity.

2.7. Ovule degeneration and style abscission

Ovule degeneration is associated with the presence of callose in their chalazal region, whose fluorescence can be observed by aniline blue staining (Mesejo et al., 2006; Zhang et al., 2018). To assess the influence of temperature on ovule life span, 20 ovules of the 'Fortune' mandarin for each temperature-day combination were isolated from the previously stained ovaries. The isolated ovules were squashed to clearly observe fluorescence without other surrounding tissues interacting.

In order to analyse the temperature effect on style abscission, 10 flowers were tagged on the day of anthesis in the 'Fortune' mandarin trees under the four temperature regimes of the study, and the changes in these flowers were monitored daily for 10 days. The day when the abscission line appeared and its distance from the style-ovary junction were recorded.

2.8. Statistical analyses

Data were confirmed to fit the normal distribution and outlier values based on box plots were removed prior to further analyses. The experimental design was double factorial. Analyses of variance and LSD multiple range tests were performed using version 16.1.03 of the Statgraphics Centurion XVI statistical software package.

3. Results

3.1. Pollen grain germination (PGG) in planta

For all nine genotype-temperature combinations, pollen grain germinated and pollen tubes grew between the finger-like papillae of the stigma surface accessing inside stigma (Fig. 2). Both genotype and temperature, as well as the genotype-temperature interactions, had a

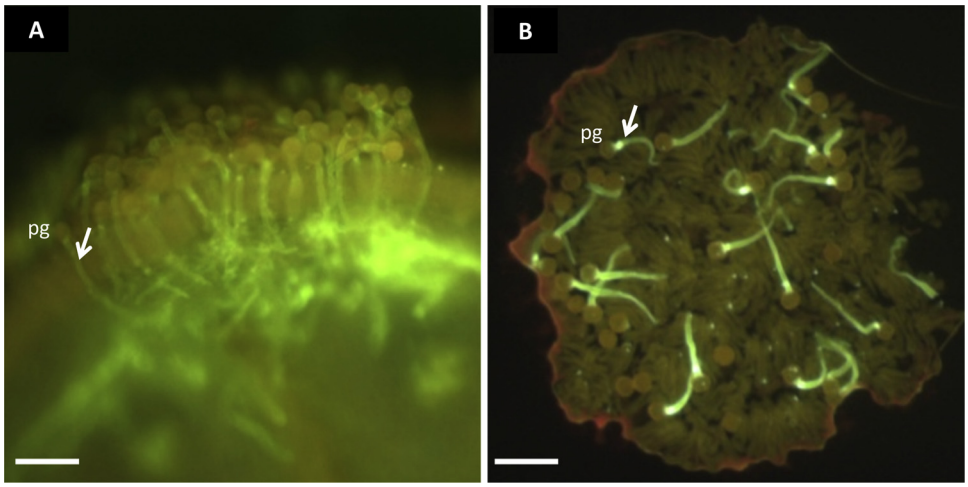

Fig. 2. *In planta* pollen grain germination of 'Clemenules' clementine on the stigma surface of 'Fortune' mandarin 24 h after pollination at 10 °C. (A) Accumulation of the germinated pollen grains and pollen tubes growing between the finger-like papillae of the stigma surface (Section 0 in Fig. 1). (B) Squash of the stigma surface on which pollen grains and pollen tubes can be individually observed. Pollen tubes are marked by an arrow; pg: pollen grain. Stigma surface stained with aniline-blue. Scale bar: 100 µm.

significant effect on PGG *in planta*, as the ANOVA revealed (Supplementary Material).

Results were expressed as a percentage of the germinated pollen grains (Table 1). A different behaviour was revealed among genotypes and was temperature-dependent. In 'Pineapple' sweet orange, the lowest percentage was observed at 10 °C, while no significant differences were detected between 20 °C and 30 °C. In 'Clemenules' clementine, differences between 20 °C and 30 °C were observed for PGG, while no significant differences were noted between these two temperatures and 10 °C. For 'Ichang' papeda, the lowest percentage was 30 °C, and no significant differences were found between 10 °C and 20 °C (Table 1).

The comparison made between genotypes at all three temperatures showed significant differences at both 10 °C and 20 °C. At these temperatures, 'Pineapple' sweet orange always displayed the lowest percentage, 'Clemenules' clementine obtained intermediate values and the highest percentage went to 'Ichang' papeda. Conversely, no differences were observed between genotypes at 30 °C (Table 1).

3.2. Pollen tube growth (PTG) dynamics in planta

The syncarpous gynoecium of the 'Fortune' mandarin is composed of 9–15 carpels fused together. Each carpel has an independent stylar canal starting from a common stigma surface that leads to a locule in the ovary. Each locule contains 4 ± 1 ovules, resulting in an average of 45 ovules per ovary (data not shown). The whole pistil length measured an average of 8.5 mm with 1.4 ± 0.3 mm for stigmas, 4.9 ± 0.7 mm for styles and 2.2 ± 0.4 mm for ovaries (Fig. 1).

In our experiments, high PGG rates were produced. Although the initiation of stylar canals takes place on the upper stigma, we observed many pollen tubes to grow outside stylar canals (Fig. 3A). From the stigma-style junction, the number of pollen tubes growing outside the stylar canals decreased (Fig. 3B); and along the lower half of the style (Fig. 3C), only pollen tubes growing inside stylar canals were observed in all the genotype-temperature combinations, which were subsequently able to reach the locules (Fig. 3D).

Regarding the quantification of PTG, the three pollen genotypes showed massive PTG in the stigma at the four studied temperatures, which decreased along the style and depended differently on both genotype and temperature (Fig. 4). As a result, the ANOVA revealed significant differences among temperature, genotype and their interaction in the maximum number of pollen tubes observed at the style-ovary junction (Supplementary Material).

In 'Pineapple' sweet orange (Fig. 4A), the number of pollen tubes lowered from the stigma-style junction at 10 °C and 30 °C, and many pollen tubes (more than 100) were observed between sections 0 and 3 at 20 °C and under FC. At the style-ovary junction (Table 2), no pollen tubes were observed at 10 °C, whereas fewer pollen tubes were noted at 30 °C compared to 20 °C and the FC. In 'Clemenules' clementine (Fig. 4B), the reduction in the number of pollen tubes started at the stigma-style junction at 30 °C, while many pollen tubes were observed between sections 0 and 3 at 10 °C, 20 °C and for the FC. At the style-ovary junction (Table 2), the maximum number of pollen tubes was significantly lower at 10 °C and 30 °C than at 20 °C and under FC, similarly to 'Pineapple' genotype. For 'Ichang' papeda, a large amount of pollen tubes was observed between sections 0 and 4–5 at 30 °C and under FC, respectively, and down to the middle of the style at 10 °C and 20 °C (Fig. 4C). At the style-ovary junction (Table 2), major differences were observed between the lowest value at 30 °C and the highest one at 10 °C, whereas intermediate values were recorded at 20 °C and under FC.

The days elapsing from pollination to the maximum number of pollen tubes observed at the style-ovary junction differed depending on both genotype and temperature. This timeframe was three days in 'Ichang' papeda at 20 °C and 30 °C and in 'Clemenules' clementine at 30 °C, four days in 'Pineapple' sweet orange at 20 °C and 30 °C and in 'Clemenules' clementine at 20 °C, and five, six and seven days in 'Ichang' papeda, 'Clemenules' clementine and 'Pineapple' sweet orange, respectively, under FC, and was 10 days in 'Clemenules' clementine and 'Ichang' papeda at 10 °C.

3.3. Pollen tube growth (PTG) kinetics in planta

The fixation of samples every day from pollination allowed us to analyse the daily progression of pollen tubes from the stigma surface through the pistil. Significant genotype and temperature effects on the section reached by pollen tubes were found on the first three days after pollination, while the effect on day four was predominantly temperature-dependent. From day five, pollen tubes reached the ovaries at

Table 1
Influence of temperature and genotype on the percentage of pollen grain germination *in planta*.

Genotypes	10 °C	20 °C	30 °C
'Pineapple'	42 ± 3.6 (a_p ; a_{10})	51 ± 6.9 (b_p ; a_{20})	54 ± 7.7 (b_p ; a_{30})
'Clemenules'	64 ± 3.4 (ab_c ; b_{10})	70 ± 6.3 (b_c ; b_{20})	60 ± 2.3 (a_c ; a_{30})
'Ichang'	81 ± 2.3 (b_i ; c_{10})	84 ± 7.3 (b_i ; c_{20})	61 ± 2.8 (a_i ; a_{30})

The percentage of germinated pollen grains is given as the mean ± SD (n = 5). In brackets, significant differences between temperatures for the same genotype and between genotypes for the same temperature are indicated by different letters in the first and second positions, respectively (p = 0.05, Fisher LSD). For each genotype-temperature combination, the initial letters of the genotype and temperature value are added as a subscript to make viewing easy.

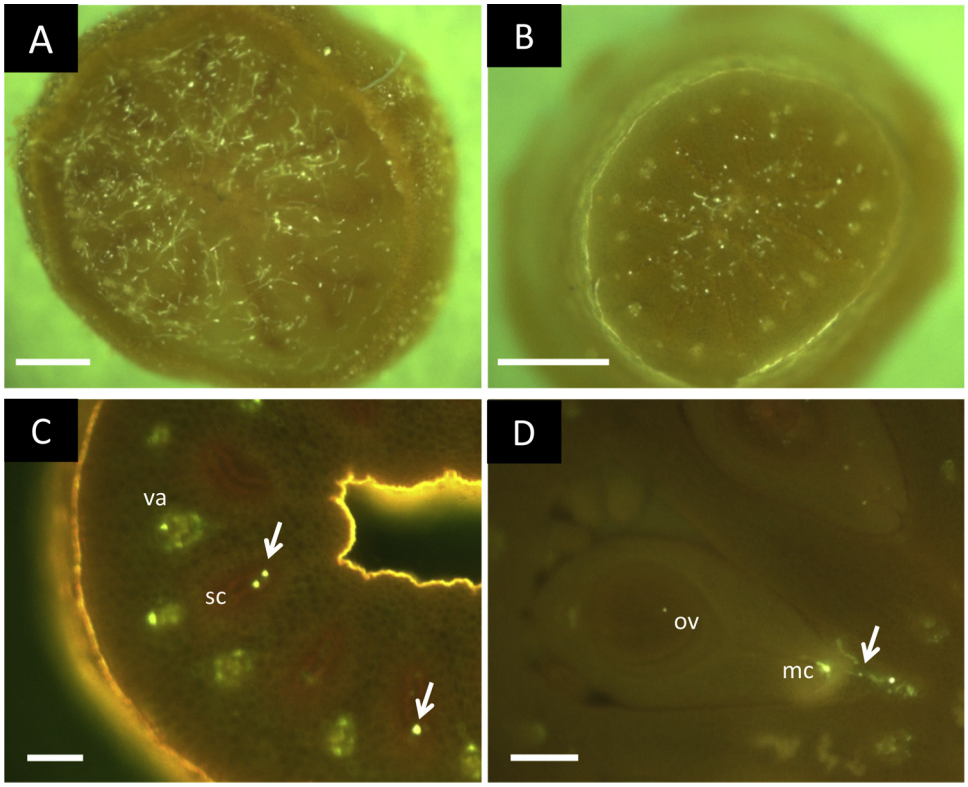

Fig. 3. Cross-sections showing pollen tube growth in the 'Fortune' mandarin pistil. (A) Massive pollen tube growth throughout the middle section of the stigma. (B) Pollen tubes growth in the upper style. (C) pollen tubes growing inside the stylar canals in the middle section of the style. (D) Pollen tube accessing the ovule through the micropyle. Figures A, B, C and D correspond to Sections 1–3–6 and 12 in Fig. 1, respectively. Pollen tubes are marked by an arrow; va: vascular axis; sc: stylar canal; ov: ovule; mc: micropyle. Sections of the pistil stained with aniline-blue. Scale bar: A,B = 500 µm; C,D = 100 µm.

20 °C, 30 °C and under FC regardless of genotype, whereas differences were genotype- and day-dependent at 10 °C (Fig. 5 and Supplementary Material).

The pollen tubes of the three male genotypes displayed a similar behaviour by reaching more basal pistil sections on a daily basis at higher temperatures, which resulted in a shorter time for pollen tubes to reach the ovary. At 30 °C, pollen tubes reached the bottom of the style two days after pollination, whereas at 20 °C they took one more day regardless of genotype (Fig. 5). However, under FC 'Ichang' papeda needed four days to get to the bottom of the style, whereas 'Pineapple' and' Clemenules' reached it on day five (Fig. 5).

The biggest differences were observed at 10 °C, as only the 'Ichang' papeda pollen tubes reached the bottom of the style in 10 days, whereas no 'Pineapple' and 'Clemenules' pollen tubes arrived at the style-ovary junction. 'Clemenules' pollen tubes reached pistil section 9, whereas 'Pineapple' pollen tubes arrived only at section 4 (Fig. 5).

3.4. Stigmatic receptivity

During the 10 experimental days, the reduction observed in germinated pollen grains and the quantity of pollen tubes growing across the middle section of the stigma evidenced a reduction in stigmatic receptivity, which was noticeably influenced by temperature (Fig. 6, Supplementary Material).

The flowers that pollinated at anthesis showed 61% of germinated pollen grains at 30 °C, and more than 80% did so at 10 °C, 20 °C and under FC, and many pollen tubes were observed to grow inside the stigma. At 30 °C, a rapid drop in both the germinated pollen grains and

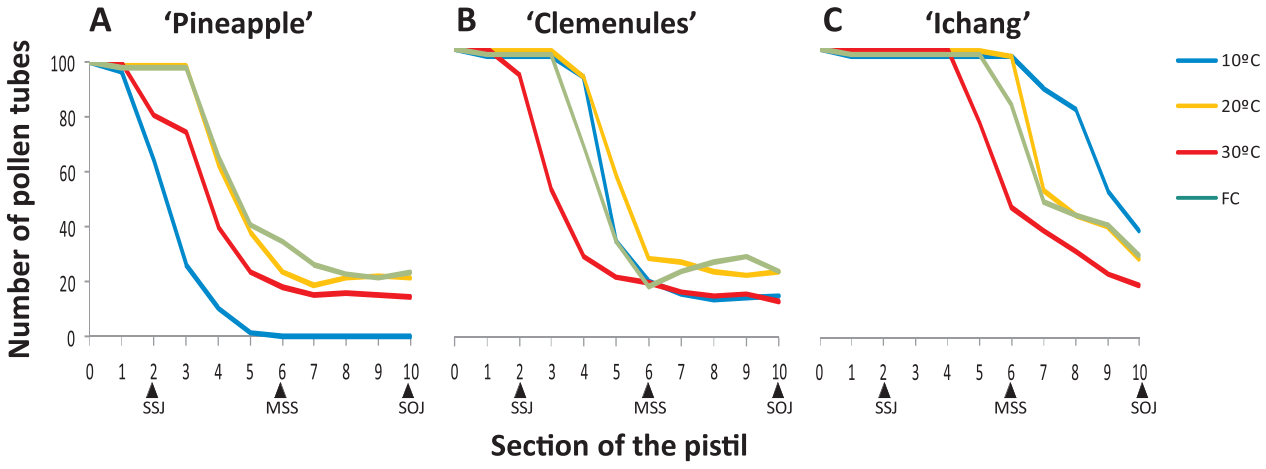

Fig. 4. Pollen tubes growth dynamics for all three crosses evaluated in the four temperature regimes. Dynamics is expressed as the average of the five maximum values of the number of pollen tubes observed in each cross-section for 10 days after pollination for (A) 'Pineapple' sweet orange; (B) 'Clemenules' clementine; and (C) 'Ichang' papeda. More than 100 pollen tubes observed in one slice were recorded as 100. The X-axis shows the sections of the pistil (displayed in Fig. 1). As a reference, the stigma-style junction (SSJ), middle section of the style (MSS) and style-ovary junction (SOJ) are pointed out below the corresponding pistil section.

Table 2
Maximum number of pollen tubes of the genotypes studied at different temperatures at the style-ovary junction of 'Fortune' mandarin pistils for 10 days after pollination.

Genotypes	10 °C	20 °C	30 °C	Field Conditions
'Pineapple'	0 ± 0.0 (a_p ; a_{10})	22 ± 2.7 (c_p ; a_{20})	15 ± 1.7 (b_p ; ab_{30})	24 ± 1.1 (c_p ; a_{FC})
'Clemenules'	14 ± 2.0 (a_c ; b_{10})	23 ± 4.3 (b_c ; a_{20})	12 ± 1.7 (a_c ; a_{30})	23 ± 1.8 (b_c ; a_{FC})
'Ichang'	37 ± 6.0 (c_i ; c_{10})	27 ± 8.4 (ab_i ; a_{20})	18 ± 5.2 (a_i ; b_{30})	28 ± 6.8 (bc_i ; a_{FC})

The maximum number of pollen tubes is given as mean ± SD (n = 5). In brackets, significant differences between temperatures for the same genotype and between genotypes for the same temperature are indicated by different letters in the first and second positions, respectively (p = 0.05 Fisher LSD). For each genotype-temperature combination, the initial letters of the genotype and temperature value are added as a subscript to make viewing easy.

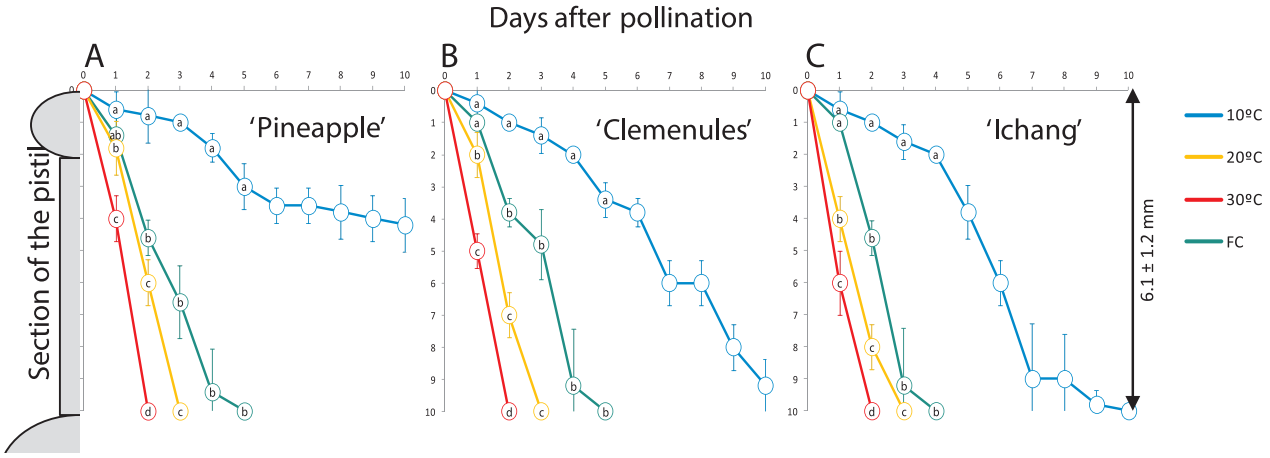

Fig. 5. Pollen tubes growth kinetics for all three crosses evaluated in the four temperature regimes. The results are expressed as the pistil section reached daily by pollen tubes (mean ± SD) for (A) 'Pineapple' sweet orange; (B) 'Clemenules' clementine; and (C) 'Ichang' papeda. Different letters in circles indicate significant differences between temperatures for the same genotype and day (0.05% Fisher LSD). Pistil is represented on the Y-axis to easily view the results, and the longitude (mean ± SD) from the stigma surface to the bottom style is shown to the right of the figure.

the quantity of pollen tubes growing along the stigma took place and resulted in a 13% of the germinated pollen grains to be coupled with a few pollen tubes growing in the stigmas that pollinated 3 days after anthesis. For the other three temperatures, although the reduction in the germinated pollen grains gradually occurred on the 10 experimental days, no pollen tubes growing along the stigma were observed in the flowers pollinated 7, 9 and 10 days after anthesis at 20 °C, under FC and 10 °C, respectively (Fig. 6). Thus the germination that took place during these days seemed non-effective in fecundation terms.

The results of this experiment indicate that stigmatic receptivity was strongly influenced by high temperature, and notably lowered the percentage of the germinated pollen grains and the number of pollen tubes growing in the middle section of the stigma on the first three days after anthesis. In contrast, cold temperatures (10 °C) prolonged the stigmatic receptivity period, with more than 30% of the pollen tubes germinated and many pollen tubes (50 ± 10) growing in the middle section of the stigma eight days after anthesis.

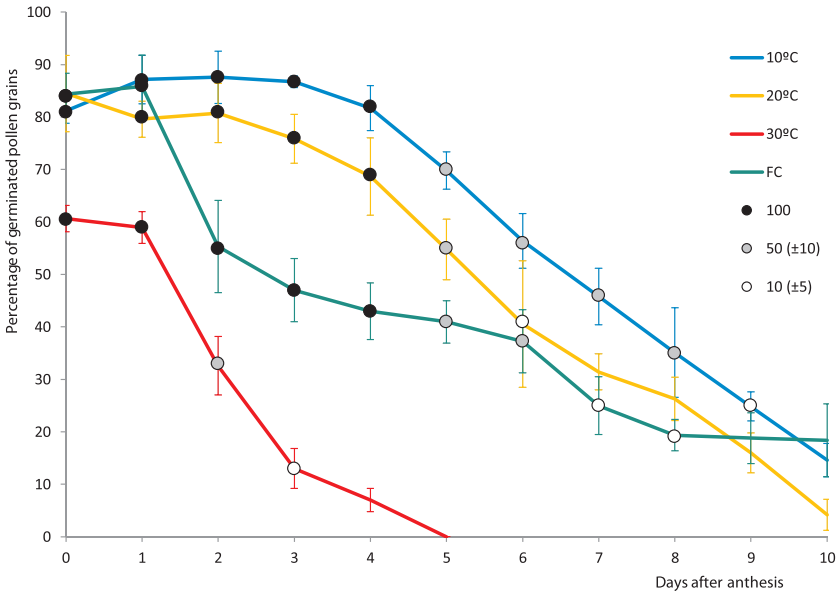

Fig. 6. Receptivity of the 'Fortune' mandarin stigmas pollinated with 'Ichang' papeda, expressed as a percentage of pollen grain germination (mean ± SD on the Y-axis) and quantity of pollen tubes observed in the middle section of the stigma (circles) from the day of anthesis (0 on the X-axis) until 10 days after anthesis at 10 °C, 20 °C, 30 °C and under the field conditions (FC). The LSD multiple range test for the variable number of pollen tubes growing in the middle section of the stigma for each temperature identified four homogeneous groups, represented with different filled circles: black represents more than 100 pollen tubes growing in the middle section of the stigma, grey denotes 50 and white indicates 10 (mean ± SD). No circle means that zero pollen tubes were observed in the middle section of the stigma.

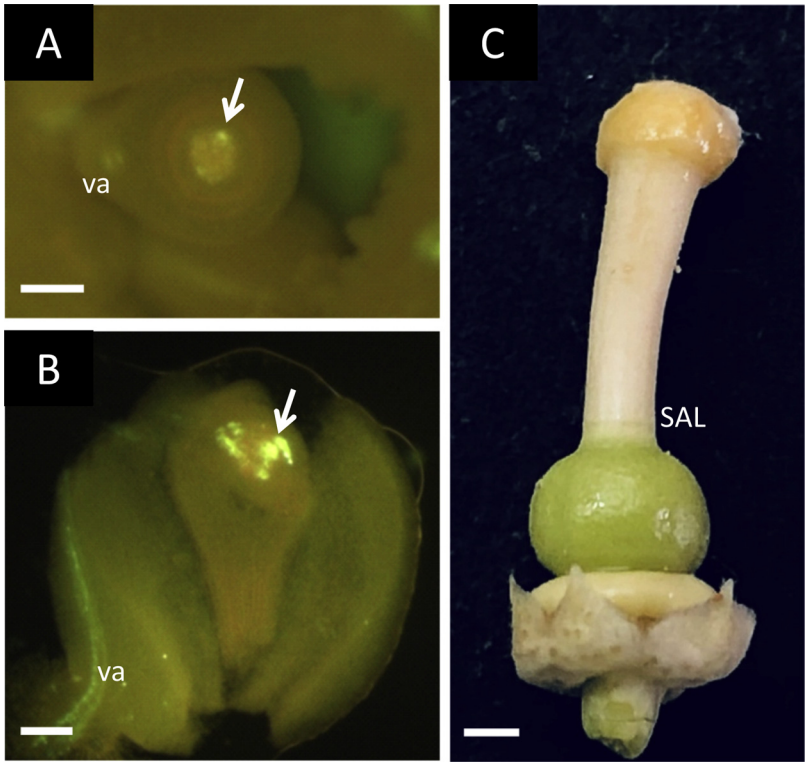

Fig. 7. 'Fortune' mandarin ovule degeneration and style abscission five days after anthesis at 30 °C. (A) cross-section of one ovule with degeneration symptoms; (B) Ovule squashed with the presence of strong fluorescence, which indicates the degeneration process; (C) Pistil with the appearance of the style abscission line (SAL). Arrows indicate ovule degeneration fluorescence. va: vascular axis. (A,B) Ovules stained with aniline-blue. Scale bar: A,B = 100 µm; C = 1000 µm.

3.5. Ovule degeneration and stylar abscission

The collected data about ovule degeneration and the appearance of the style abscission line (SAL) on 10 consecutive days after anthesis in the four studied temperature regimes showed that temperature significantly influenced both processes (Figs. 7–8, Supplementary Material). Ovule degeneration is associated with the presence of callose fluorescence (Fig. 7A–B) and the first symptoms (with 10% degenerating ovules) were observed four days after anthesis at 30 °C, five days at 20 °C and seven days under FC. At 10 °C, no ovule degeneration took place during the 10-day observation period. Conversely at 30 °C, all the ovules were degenerated on day seven after anthesis, while the percentage of ovule degeneration was 77% and 20%, respectively, at 20 °C and under FC (Fig. 8A).

Regarding stylar abscission (Fig. 7C), no SAL was observed until four days after anthesis at 30 °C, six days at 20 °C and seven days under FC. At 10 °C, no SAL was observed during the 10-day observation period. At 30 °C and five days after anthesis, the SAL was observed in 60% of the styles and all the styles showed the SAL on day seven after anthesis. At 20 °C and under FC, about 40% of the styles showed the SAL on day seven and eight after anthesis, respectively, whereas the percentage of SAL was 90% and 80%, respectively, after 10 days (Fig. 8B). No differences in the distance from the SAL to the ovary were observed between the styles subjected to different temperatures (data not shown).

These results revealed that the first ovule degeneration symptoms occurred earlier than the first appearance of the SAL. The biggest difference was recorded under FC, with notable differences between the ovule degeneration and SAL percentages. For example, 10 days after anthesis, the ovule degeneration and SAL percentages were more than 20% and 80%, respectively, but no differences were observed at low temperatures (Fig. 8).

4. Discussion

Temperature stress is a key parameter in the progamic phase in plants. Our results offer accurate knowledge about the influence of temperature in the progamic phase by dissecting the effects of both temperature and genotype on the male donor and the effect of temperature on the female recipient.

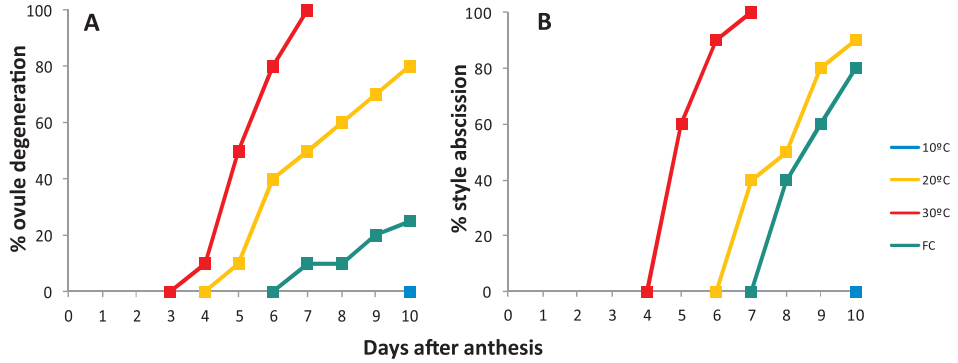

Fig. 8. Pistil degeneration of 'Fortune' mandarin from 0 to 10 days after anthesis at 10 °C, 20 °C, 30 °C and under FC. Values are expressed as (A) a percentage of ovule degeneration and (B) a percentage of the pistils with a style abscission line.

4.1. Temperature and genotype influence on the male donor: pollen grain germination (PGG) and pollen tube growth (PTG)

For male parents, our results showed the influence of temperature and genotype, and their interaction, on both PGG and PTG, as well as differences in the optimal temperature for PGG and PTG kinetics, which fall in line with the independence of these processes as reported previously (Distefano et al., 2012; Kakani et al., 2002; Mckee and Richards, 1998). The methodology formerly reported to evaluate PGG is based on *in vitro* tests. Distefano et al. (2012) analysed PGG *in vitro* of different citrus species from 10 °C to 30 °C, and found that no PGG was produced at 10 °C. However, we observed PGG at 10 °C for the three studied genotypes. This discrepancy may be due to the fact that we analysed PGG directly on the stigma surface *in planta* instead of testing PGG *in vitro*. Differences in the pollen germination rates between *in vitro* and *in vivo* tests have been reported in tobacco (Shivanna et al., 1991) and sweet cherry (Hormaza and Herrero, 1999), and suggest that *in vitro* germination media do not provide the optimal conditions offered by the stigma. In contrast, the pollen grain germination analysis performed *in planta* is a more accurate method of testing actual pollen performance because germination occurs in the stigmatic secretion, which is composed of lipids, polysaccharides and proteins (Cresti et al., 1982; Rejón et al., 2014, 2013), and plays an important role in pollen adhesion and germination (Distefano et al., 2011).

Among the 12 analysed pollen-temperature combinations, the biggest differences in both PGG and PTG were observed between 'Pineapple' sweet orange and 'Ichang' papeda at 10 °C. The worst pollen performance observed in 'Pineapple' sweet orange, in contrast to the best performance noted in 'Ichang' papeda, may be associated with the previously reported high sensitivity to frost of 'Pineapple' sweet orange and the cold-resistance of 'Ichang' papeda (Hodgson, 1967). However, no significant differences were noted between genotypes at the warmest temperature in our study (30 °C).

Bono et al. (2000) reported the number of seeds in the 'Fortune x Clemenules' cross under FC which was 26 seeds/fruit on average. This is consistent with our observation of the 23 pollen tubes of the 'Clemenules' clementine at the style-ovary junction of 'Fortune' mandarin under FC. This finding supports the possibility that the pollen tubes at the style base could be used to estimate seed quantity in the sexual hybridisations among citrus species, provided that no mechanism intervenes to jeopardise embryo development and seed formation.

The average temperature under FC (18.5 °C) was similar to 20 °C in the growth chamber. However, the PTG kinetics results obtained for these temperatures significantly differed. This could be due to a drop in temperature around 10 °C for several hours a day in the FC regime, which slowed down the PTG kinetics. Despite differences in the PTG kinetics, no differences were observed in the maximum number of pollen tubes that reached the style-ovary junction, which indicated that in fertilization terms, PTG behaviour may be inferred by evaluating constant temperature regimes with similar temperature averages to those of the studied FC.

In other species, the response of PGG and PTG to temperature stress has been used to screen genotypes that are tolerant to both high and cold temperatures, and to also transfer this tolerance to offspring (Domínguez et al., 2005; Kakani et al., 2005, 2002; Liu et al., 2006; Zamir et al., 1982). Previous results suggest that temperature stress in the reproductive phase produces a natural selection of the best-adapted pollen tubes (Hedhly et al., 2009), and a significant correlation between male gametophyte and sporophyte behaviour for temperature stress has been confirmed (Hebbar et al., 2018; Hedhly, 2011; Hormaza and Herrero, 1992). Our experimental design allowed us to observe the daily progress of PTG (kinetics and dynamics) and thus showed a timeframe for each genotype between the day when the first pollen tubes reached the ovary (which could correspond to the best-adapted gametes to the prevailing temperature) and the day on which the maximum pollen tubes reached the ovary. This observation opens up the possibility to limit fertilisation to the gametes displaying the best behaviour upon temperature stress by removing part of the pistil (stigma and/or style) soon after the first pollen tubes reach the ovary. Previous studies have reported seed formation from early removed stigmas in rice (Chen et al., 2008). In citrus, our preliminary observations indicate that pollen tubes are capable of fertilising ovules in early removed pistils (stigma and/or style), but the ability to obtain seeded fruits after this procedure remains unknown. This method could provide an opportunity to explore gametophytic selection pressure in the progamic phase, in addition to parental selection based on our above-mentioned results.

Knowledge about how temperature affects pollen performance can be useful for better planning the number of pollinations in breeding programmes based on sexual hybridisations, and to also improve hybrid production by choosing the most favourable time and location to perform pollination depending on temperature forecasts. Our breeding programme in Spain includes three locations available to perform pollinations: a region located in the province of Huelva on the Atlantic coast with warm temperatures; another area in Valencia characterised by more moderate temperatures; and a third one north of the province of Castellón characterised by colder temperatures during the flowering period of citrus fruits between April and May. Therefore, these results can be applied to perform further pollinations under the most favourable conditions.

4.2. Temperature influence on the female recipient: stigma receptivity, ovule degeneration and style abscission

Stigmatic receptivity and ovule degeneration are key points in regulating the interaction between male and female reproductive phases, and have important consequences on the EPP. Both issues are influenced by environmental conditions and temperature has a clear effect on the modulation of these processes (Cerović et al., 2000; Lora et al., 2011). In citrus, information about stigma receptivity and ovule degeneration is scarce and non-existent regarding the influence of temperature on them. Regarding style abscission, previous visual observations have suggested that high temperatures accelerate this process (Estornell et al., 2016). Indeed, the above authors considered that the differences in the style abscission timing of *C. sinensis* and *C. bergamia* between two consecutive flowering seasons were due to the distinct average temperatures between both seasons.

In all temperature regimes dealt with in this study, pistil senescence started with loss of stigmatic receptivity, followed by ovule degeneration and finally by style abscission from the ovary. Our results showed that temperature had a clear effect on pistil degeneration in 'Fortune' mandarin. Warm temperature regimes shortened the stigmatic receptivity period and the ovule life span, and anticipated style abscission from the ovary, whereas the cold temperature regime had the opposite effect.

This work provides new information about how temperature affects the stigmatic receptivity in the progamic phase in citrus. The percentage of germinated pollen grains progressively lowered as flowers were pollinated on subsequent post-anthesis days. Likewise, we noticed that the number of pollen tubes growing in the stigma decreased. In all temperature regimes in this study, the growth ability of pollen tubes is lost before the ability of pollen grain to germinate. Similar results have been found in sweet cherry (Hedhly et al., 2003) and peach (Hedhly et al., 2005a, 2005b).

Under FC, the drop in stigmatic receptivity in the flowers pollinated 7–8 days after anthesis and the total stigmatic degeneration in the flowers pollinated 9–10 days after anthesis, according to our results, agree with the seed set (7 seeds in the flowers pollinated 8 days after anthesis and no seeds in the flowers pollinated 10 days after anthesis) resulting from delaying pollinations using the 'Clemenules x Fortune' cross under FC (Mesejo et al., 2007). In addition, 20% of the ovules degenerated 10 days after anthesis under the FC similarly to those

observed by the same authors in 'Clemenules' under FC.

As we have shown, the stigma of 'Fortune' mandarin is receptive at anthesis. This is of much practical value for citrus breeding programmes based on sexual hybridisation since effective pollination can be performed when flowers are at anthesis, which facilitates such process. This also occurs in other woody species like peach, sweet cherry and kiwi (Sanzol and Herrero, 2001), whereas post-anthesis maturation is required for optimal stigma receptivity in almond (Yi et al., 2006). In addition, knowledge about the amount of time pollination can be delayed from anthesis (which depends on temperature, as we have shown in this piece of research) without significantly reducing the quantity of obtained seeds allows flexibility in decision-making during the flowering period in breeding programmes.

4.3. Pollen and pistil synchronic response to temperature stress enable mating

Our method consisted of cultivating whole plants in culture chambers and observing the cross-sections of the pistils fixed daily for 10 days from pollination. This method enabled us to acquire more comprehensive knowledge about the pollen-pistil interaction in the progamic phase. In this study, pollen tubes were able to reach the ovules in all the evaluated combinations on the 10 experimental days, except for the 'Fortune' x 'Pineapple' cross at 10 °C combination. However, as neither ovule degeneration nor pistil abscission were observed at this temperature, it could be possible that pollen tubes of 'Pineapple' sweet orange reached ovaries of 'Fortune' mandarin after the 10 days of the experiment. Our results show that when performing pollinations at anthesis, the studied crosses were able to successfully perform the progamic phase with temperature changes by maintaining the male-female synchrony described as being necessary for successful mating (Herrero, 2003). This plasticity is reflected by the fact that citrus plants are cultivated in 147 countries around the world (FAOSTAT, 2017), and at between approximately latitudes of 40 °N and 40 °S that comprise tropical, subtropical and colder areas. Knowledge about the influence of temperature in the progamic phase on citrus plants is of much interest as climate change during the flowering season can alter the progamic phase and, consequently, the reproductive process.

Temperature affects both PGG and PTG dynamics and kinetics because high and low temperatures respectively accelerate and decelerate these processes. Regarding the female parent, high temperatures accelerate stigma and ovule development, which results in a shorter period of stigmatic receptivity and a shorter ovule life span, whereas low temperatures extend both processes and, thus, also the EPP. These results allow us to suggest that temperature stress from 10 °C to 30 °C has a complementary effect on both male and female parents by accelerating or decelerating the progamic phase. Nevertheless, as highlighted by Hedhly et al. (2009) and as observed herein, the response to stress caused by temperature is genotype-dependent. This means that variations within the optimal range for each genotype may alter, or even interrupt the reproduction process and lead to a low or null seed content and/or fruit set. In citrus fruits, especially mandarins, parthenocarpy is a common phenomenon and seed production is not necessary to obtain good yields in most varieties. However, this decoupling would hamper large populations being obtained in breeding programmes based on sexual hybridisations, where it is necessary to recover large numbers of seeds for specific male and female combinations.

5. Conclusions

Despite the strong influence of temperature in the progamic phase, the evaluated crosses are capable of responding to environmental changes and ensuring good fertilisation levels.

The results of this paper can be useful for improving pollination efficiency and adapting breeding programmes to the temperature forecasts during the pollination period. Our results also suggest that pollen performance-based screening may be a useful strategy to select better adapted citrus genotypes to different environmental conditions, and also to explore gametophytic selection within genotypes.

In future research, it would be relevant to investigate the influence of temperature and genotype during gametogenesis. If coupled with the results obtained for the progamic phase, such investigation could be useful for enhancing the efficiency of citrus breeding programmes based on sexual hybridisation; in particular, those whose aim is to obtain new varieties that can adapt to both colder areas and current areas in the process of becoming warmer as a result of global climate change.

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CRediT authorship contribution statement

Rafael Montalt: Conceptualization, Methodology, Writing - original draft. **José Cuenca:** Methodology, Writing - review & editing. **María Carmen Vives:** Writing - review & editing. **Luis Navarro:** Conceptualization, Writing - review & editing. **Patrick Ollitrault:** Conceptualization, Writing - review & editing. **Pablo Aleza:** Conceptualization, Validation, Writing - review & editing, Project administration.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2019.103806.

References

Acar, I., Kakani, V.G., 2010. The effects of temperature on in vitro pollen germination and pollen tube growth of Pistacia spp. Sci. Hortic. (Amsterdam) 125, 569–572. https://doi.org/10.1016/j.scienta.2010.04.040.

Aleza, P., Juárez, J., Cuenca, J., Ollitrault, P., Navarro, L., 2012a. Extensive citrus triploid hybrid production by 2x × 4x sexual hybridizations and parent-effect on the length of the juvenile phase. Plant Cell Rep. 31, 1723–1735. https://doi.org/10.1007/s00299-012-1286-0.

Aleza, P., Juárez, J., Cuenca, J., Ollitrault, P., Navarro, L., 2010. Recovery of citrus triploid hybrids by embryo rescue and flow cytometry from 2x × 2x sexual hybridisation and its application to extensive breeding programs. Plant Cell Rep. 29, 1023–1034. https://doi.org/10.1007/s00299-010-0888-7.

Aleza, P., Juárez, J., Hernández, M., Ollitrault, P., Navarro, L., 2012b. Implementation of extensive citrus triploid breeding programs based on 4x × 2x sexual hybridisations. Tree Genet. Genomes 8, 1293–1306. https://doi.org/10.1007/s11295-012-0515-6.

Aloni, B., Peet, M., Pharr, M., Karni, L., 2001. The effect of high temperature and high atmospheric CO2 on carbohydrate changes in bell pepper (Capsicum annuum) pollen in relation to its germination. Physiol. Plant. 112, 505–512. https://doi.org/10.1034/j.1399-3054.2001.1120407.x.

Beppu, K., Suehara, T., Kataoka, I., 2001. Embryo sac development and fruit set of "Satohnishiki" sweet cherry as affected by temperature, GA3 and paclobutrazol. J. Jpn. Soc. Hortic. Sci. 70, 157–162. https://doi.org/10.2503/jjshs.70.157.

Bono, R., Soler, J., Buj, A., 2000. Parámetros de calidad de los cítricos. El problema de las semillas. Rev. Comunidad Valencia. Agrar. 7–15.

Buchholz, J.T., Blakeslee, A.F., 2006. Pollen-Tube Growth at Various Temperatures. Am. J. Bot. 14, 358. https://doi.org/10.2307/2435941.

Cerović, R., Ružić, Đ., Mićić, N., 2000. Viability of plum ovules at different temperatures. Ann. Appl. Biol. 137, 53–59. https://doi.org/10.1111/j.1744-7348.2000.tb00056.x.

Chen, S.-Q., Zhong, W., Liu, M.-X., Xie, Z.-W., Wang, H.-H., 2008. Pollen grain germination and pollen tube growth in pistil of rice. Rice Sci. 15, 125–130. https://doi.org/10.1016/S1672-6308(08)60030-X.

Coast, O., Murdoch, A.J., Ellis, R.H., Hay, F.R., Jagadish, K.S.V., 2016. Resilience of rice (Oryza spp.) pollen germination and tube growth to temperature stress. Plant Cell Environ. 39, 26–37. https://doi.org/10.1111/pce.12475.

Corlett, R.T., Westcott, D.A., 2013. Will plant movements keep up with climate change? Trends Ecol. Evol. 28, 482–488. https://doi.org/10.1016/j.tree.2013.04.003.

Cresti, M., Ciampolini, F., van Went, J.L., Wilms, H.J., 1982. Ultrastructure and histochemistry of Citrus limon (L.) stigma. Planta 156, 1–9. https://doi.org/10.1007/

BF00393436.
Distefano, G., Gentile, A., Hedhly, A., La Malfa, S., 2018. Temperatures during flower bud development affect pollen germination, self-incompatibility reaction and early fruit development of clementine (Citrus clementina Hort. ex Tan.). Plant Biol. https://doi.org/10.1111/plb.12656.
Distefano, G., Gentile, A., Herrero, M., 2011. Pollen-pistil interactions and early fruiting in parthenocarpic citrus. Ann. Bot. 108, 499–509. https://doi.org/10.1093/aob/mcr187.
Distefano, G., Hedhly, A., Las Casas, G., La Malfa, S., Herrero, M., Gentile, A., 2012. Male-female interaction and temperature variation affect pollen performance in Citrus. Sci. Hortic. (Amsterdam) 140, 1–7. https://doi.org/10.1016/j.scienta.2012.03.011.
Domínguez, E., Cuartero, J., Fernández-Muñoz, R., 2005. Breeding tomato for pollen tolerance to low temperatures by gametophytic selection. Euphytica 142, 253–263. https://doi.org/10.1007/s10681-005-2042-0.
Elgersma, A., Stephenson, A.G., Nijs, A.P.M., 1989. Effects of genotype and temperature on pollen tube growth in perennial ryegrass (Lolium perenne L.). Sex. Plant Reprod. 2, 225–230. https://doi.org/10.1007/BF00195583.
Erner, Y., Shomer, L., 1996. Morphology and anatomy of stems and pedicels of spring flush shoots associated with citrus fruit set. Ann. Bot. 77, 537–545.
Estornell, L.H., Gómez, M.D., Pérez-Amador, M.A., Talón, M., Tadeo, F.R., 2016. Secondary abscission zones: Understanding the molecular mechanisms triggering stylar abscission in citrus. Acta Hortic. 1119, 65–72. https://doi.org/10.17660/ActaHortic.2016.1119.9.
FAOSTAT [WWW Document], 2017. URL http://www.fao.org/faostat/en/#data/QC (Accessed 17 May 2019).
Gao, Y.Bin, Wang, C.L., Wu, J.Y., Zhou, H.S., Jiang, X.T., Wu, J., Zhang, S.L., 2014. Low temperature inhibits pollen tube growth by disruption of both tip-localized reactive oxygen species and endocytosis in Pyrus bretschneideri Rehd. Plant Physiol. Biochem. 74, 255–262. https://doi.org/10.1016/j.plaphy.2013.11.018.
Hebbar, K.B., Rose, H.M., Nair, A.R., Kannan, S., Niral, V., Arivalagan, M., Gupta, A., Samsudeen, K., Chandran, K.P., Chowdappa, P., Vara Prasad, P.V., 2018. Differences in in vitro pollen germination and pollen tube growth of coconut (Cocos nucifera L.) cultivars in response to high temperature stress. Environ. Exp. Bot. 153, 35–44. https://doi.org/10.1016/j.envexpbot.2018.04.014.
Hedhly, A., 2011. Sensitivity of flowering plant gametophytes to temperature fluctuations. Environ. Exp. Bot. 74, 9–16. https://doi.org/10.1016/j.envexpbot.2011.03.016.
Hedhly, A., Hormaza, J.I., Herrero, M., 2009. Global warming and sexual plant reproduction. Trends Plant Sci. 14, 30–36. https://doi.org/10.1016/j.tplants.2008.11.001.
Hedhly, A., Hormaza, J.I., Herrero, M., 2005a. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. Plant Biol. 7, 476–483. https://doi.org/10.1055/s-2005-865850.
Hedhly, A., Hormaza, J.I., Herrero, M., 2005b. Influence of genotype-temperature interaction on pollen performance. J. Evol. Biol. 18, 1494–1502. https://doi.org/10.1111/j.1420-9101.2005.00939.x.
Hedhly, A., Hormaza, J.I., Herrero, M., 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, Prunus avium (Rosaceae). Am. J. Bot. 91, 558–564. https://doi.org/10.3732/ajb.91.4.558.
Hedhly, A., Hormaza, J.I., Herrero, M., 2003. The effect of temperature on stigmatic receptivity in sweet cherry (Prunus avium L.). Plant Cell Environ. 26, 1673–1680. https://doi.org/10.1046/j.1365-3040.2003.01085.x.
Herrero, M., 2003. Male and female synchrony and the regulation of mating in flowering plants. Philos. Trans. R. Soc. B Biol. Sci. 358, 1019–1024. https://doi.org/10.1098/rstb.2003.1285.
Hodgson, R.W., 1967. Horticultural varieties of Citrus. In: Reuther, W., Webber, H., Batchelor, L. (Eds.), The Citrus Industry. Volume I. History, World Distribution, Botany, and Varieties. University of California, pp. 431–611.
Hormaza, J.I., Herrero, M., 1999. Pollen performance as affected by the pistilar genotype in sweet cherry (Prunus avium L.). Protoplasma 208, 129–135. https://doi.org/10.1007/BF01279083.
Hormaza, J.I., Herrero, M., 1992. Pollen selection. Theor. Appl. Genet 83–83, 663–672. https://doi.org/10.1007/BF00226682.
Huang, J.-H., Ma, W.-H., Liang, G.-L., Zhang, L.-Y., Wang, W.-X., Cai, Z.-J., Wen, S.-X., 2010. Effects of low temperatures on sexual reproduction of 'Tainong 1' mango (Mangifera indica). Sci. Hortic. (Amsterdam) 126, 109–119. https://doi.org/10.1016/j.scienta.2010.06.017.
Iizumi, T., Furuya, J., Shen, Z., Kim, W., Okada, M., Fujimori, S., Hasegawa, T., Nishimori, M., 2017. Responses of crop yield growth to global temperature and socioeconomic changes. Sci. Rep. 7, 7800. https://doi.org/10.1038/s41598-017-08214-4.
IPCC, 2014. Climate change 2014 synthesis report summary chapter for policymakers. Ipcc 31. https://doi.org/10.1017/CBO9781107415324.
IVIA, [WWW Document], 2019. URL http://riegos.ivia.es/datos-meteorologicos.
Jefferies, C.J., Brain, P., 1984. A mathematical model of pollen-tube penetration in apple styles. Planta 160, 52–58. https://doi.org/10.1007/BF00392465.
Jefferies, C.J., Brain, P., Stott, K.G., Belcher, A.R., 1982. Experimental systems and a mathematical model for studying temperature effects on pollen-tube growth and fertilization in plum. Plant Cell Environ. 5, 231–236. https://doi.org/10.1111/1365-3040.ep11572417.
Johansen, D., 1940. Plant Microtechniques. McGraw-Hill, New York, NY, USA.
Kakani, V.G., Prasad, P.V.V., Craufurd, P.Q., Wheeler, T.R., 2002. Response of in vitro pollen germination and pollen tube growth of groundnut (Arachis hypogaea L.) genotypes to temperature. Plant Cell Environ. 25, 1651–1661. https://doi.org/10.1046/j.1365-3040.2002.00943.x.
Kakani, V.G., Reddy, K.R., Koti, S., Wallace, T.P., Prasad, P.V.V., Reddy, V.R., Zhao, D., 2005. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Ann. Bot. 96, 59–67. https://doi.org/10.1093/aob/mci149.
Koti, S., Reddy, K.R., Reddy, V.R., Kakani, V.G., Zhao, D., 2005. Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (Glycine max L.) flower and pollen morphology, pollen production, germination, and tube lengths. J. Exp. Bot. 56, 725–736. https://doi.org/10.1093/jxb/eri044.
Koubouris, G.C., Metzidakis, I.T., Vasilakakis, M.D., 2009. Impact of temperature on olive (Olea europaea L.) pollen performance in relation to relative humidity and genotype. Environ. Exp. Bot. 67, 209–214. https://doi.org/10.1016/j.envexpbot.2009.06.002.
Lewis, D., 1942. The physiology of incompatibility in plants. I. The effect of temperature. Proc. R. Soc. B Biol. Sci. 131, 13–26. https://doi.org/10.1098/rspb.1942.0015.
Linskens, F.H., Esser, K., 1957. Über eine spezifische anfarbung der pollenschlauche im griffel und die zahl der kallospefropfen nach slbstdung und femddung. Naturwissenschaften 44, 16.
Liu, Z., Yuan, Y.L., Liu, S.Q., Yu, X.N., Rao, L.Q., 2006. Screening for high-temperature tolerant cotton cultivars by testing in vitro pollen germination, pollen tube growth and boll retention. J. Integr. Plant Biol. 48, 706–714. https://doi.org/10.1111/j.1744-7909.2006.00276.x.
Lora, J., Herrero, M., Hormaza, J.I., 2011. Stigmatic receptivity in a dichogamous early-divergent angiosperm species, Annona cherimola (Annonaceae): Influence of temperature and humidity. Am. J. Bot. 98, 265–274. https://doi.org/10.3732/ajb.1000185.
Lora, J., Herrero, M., Hormaza, J.I., 2009. The coexistence of bicellular and tricellular pollen in Annona cherimola (Annonaceae): Implications for pollen evolution. Am. J. Bot. 96, 802–808. https://doi.org/10.3732/ajb.0800167.
Luza, J.G., Polito, V.S., Weinbaum, S.A., 1987. Staminate Bloom Date and Temperature Responses of Pollen Germination and Tube Growth in Two Walnut (Juglans) Species. Am. J. Bot. 74, 1898. https://doi.org/10.2307/2443973.
Matsumoto, Y., Miyagi, M., Watanabe, N., Kuboyama, T., 2012. Temperature-dependent enhancement of pollen tube growth observed in interspecific crosses between wild Cucumis spp. and melon (C. melo L.). Sci. Hortic. (Amsterdam) 138, 144–150. https://doi.org/10.1016/j.scienta.2012.02.016.
Mckee, J., Richards, A.J., 1998. The effect of temperature on reproduction in five Primula species. Ann. Bot. 82, 359–374. https://doi.org/10.1006/anbo.1998.0697.
Mesejo, C., Martínez-Fuentes, A., Reig, C., Agustí, M., 2007. The effective pollination period in 'Clemenules' mandarin, 'Owari' Satsuma mandarin and 'Valencia' sweet orange. Plant Sci. 173, 223–230. https://doi.org/10.1016/J.PLANTSCI.2007.05.009.
Mesejo, C., Martínez-Fuentes, A., Reig, C., Rivas, F., Agustí, M., 2006. The inhibitory effect of CuSO4 on Citrus pollen germination and pollen tube growth and its application for the production of seedless fruit. Plant Sci. 170, 37–43. https://doi.org/10.1016/J.PLANTSCI.2005.07.023.
Navarro, L., Aleza, P., Cuenca, J., Juárez, J., Pina, J.A., Ortega, C., Navarro, A., Ortega, V., 2015. The mandarin triploid breeding program in Spain. Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 389–396. https://doi.org/10.17660/ActaHortic.2015.1065.48.
Nygaard, P., 1969. Studies on the Germination of Pine Pollen (Pinus mugo) in vitro. I. Growth Conditions and Effects of pH and Temperature on Germination, Tube Growth and Respiration. Physiol. Plant. 22, 338–346. https://doi.org/10.1111/j.1399-3054.1969.tb07384.x.
Pham, V.T., Herrero, M., Hormaza, J.I., 2015. Effect of temperature on pollen germination and pollen tube growth in longan (Dimocarpus longan Lour.). Sci. Hortic. (Amsterdam) 197, 470–475. https://doi.org/10.1016/j.scienta.2015.10.007.
Postweiler, K., Stösser, R., Anvari, S.F., 1985. The effect of different temperatures on the viability of ovules in cherries. Sci. Hortic. (Amsterdam) 25, 235–239. https://doi.org/10.1016/0304-4238(85)90120-7.
Prasad, P.V.V., Boote, K.J., Allen, L.H., Thomas, J.M.G., 2002. Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (Phaseolus vulgaris L.). Glob. Change Biol. 8, 710–721. https://doi.org/10.1046/j.1365-2486.2002.00508.x.
Radičević, S., Cerović, R., Nikolić, D., Đorđević, M., 2016. The effect of genotype and temperature on pollen tube growth and fertilization in sweet cherry (Prunus avium L.). Euphytica 209, 121–136. https://doi.org/10.1007/s10681-016-1645-y.
Rejón, J.D., Delalande, F., Schaeffer-Reiss, C., Carapito, C., Zienkiewicz, K., de Dios Alché, J., Isabel Rodríguez-García, M., Van Dorsselaer, A., Castro, A.J., 2014. The plant stigma exudate: a biochemically active extracellular environment for pollen germination? Plant Signal. Behav. 9https://doi.org/10.4161/psb.28274. e28274–e28274.
Rejón, J.D., Delalande, F., Schaeffer-Reiss, C., Carapito, C., Zienkiewicz, K., de Dios Alché, J., Rodríguez-García, M.I., Van Dorsselaer, A., Castro, A.J., 2013. Proteomics profiling reveals novel proteins and functions of the plant stigma exudate. J. Exp. Bot. 64, 5695–5705. https://doi.org/10.1093/jxb/ert345.
Sage, T.L., Bagha, S., Lundsgaard-Nielsen, V., Branch, H.A., Sultmanis, S., Sage, R.F., 2015. The effect of high temperature stress on male and female reproduction in plants. Feild Crop. Res. 182, 30–42. https://doi.org/10.1016/j.fcr.2015.06.011.
Sanzol, J., Herrero, M., 2001. The "effective pollination period" in fruit trees. Sci. Hortic. https://doi.org/10.1016/S0304-4238(00)00252-1.
Sato, S., Peet, M.M., Thomas, J.F., 2002. Determining critical pre- and post-anthesis periods and physiological processes in Lycopersicon esculentum Mill. exposed to moderately elevated temperatures. J. Exp. Bot. 53, 1187–1195. https://doi.org/10.1093/jexbot/53.371.1187.
Schindelin, J., Rueden, C.T., Hiner, M.C., Eliceiri, K.W., 2015. The ImageJ ecosystem: an open platform for biomedical image analysis. Mol. Reprod. Dev. 82, 518–529. https://doi.org/10.1002/mrd.22489.
Sedgley, M., 1977. The Effect of Temperature on Floral Behaviour, Pollen Tube Growth and Fruit Set in the Avocado. J. Hortic. Sci. 52, 135–141. https://doi.org/10.1080/00221589.1977.11514739.
Shivanna, K.R., Linskens, H.F., Cresti, M., 1991. Responses of tobacco pollen to high

humidity and heat stress: viability and germinability in vitro and in vivo. Sex. Plant Reprod. 4, 104–109. https://doi.org/10.1007/BF00196495.

Singer, A., Johst, K., Banitz, T., Fowler, M.S., Groeneveld, J., Gutiérrez, A.G., Hartig, F., Krug, R.M., Liess, M., Matlack, G., Meyer, K.M., Pe'er, G., Radchuk, V., Voinopol-Sassu, A.J., Travis, J.M.J., 2016. Community dynamics under environmental change: how can next generation mechanistic models improve projections of species distributions? Ecol. Modell. 326, 63–74. https://doi.org/10.1016/j.ecolmodel.2015.11.007.

Snider, J.L., Oosterhuis, D.M., Kawakami, E.M., 2011a. Diurnal pollen tube growth rate is slowed by high temperature in field-grown Gossypium hirsutum pistils. J. Plant Physiol. 168, 441–448. https://doi.org/10.1016/j.jplph.2010.08.003.

Snider, J.L., Oosterhuis, D.M., Loka, D.A., Kawakami, E.M., 2011b. High temperature limits in vivo pollen tube growth rates by altering diurnal carbohydrate balance in field-grown Gossypium hirsutum pistils. J. Plant Physiol. 168, 1168–1175. https://doi.org/10.1016/j.jplph.2010.12.011.

Soost, R.K., 1965. Incompatibility alleles in the genus Citrus. J. Am. Soc. Hortic. Sci. 87, 176–180.

Springate, D.A., Kover, P.X., 2014. Plant responses to elevated temperatures: A field study on phenological sensitivity and fitness responses to simulated climate warming. Glob. Chang. Biol. 20, 456–465. https://doi.org/10.1111/gcb.12430.

Williams, R.R., 1965. The Effect of Summer Nitrogen Applications on The Quality of Apple Blossom. J. Hortic. Sci. 40, 31–41. https://doi.org/10.1080/00221589.1965.11514118.

Yi, W., Law, S.E., McCoy, D., Wetzstein, H.Y., 2006. Stigma Development and Receptivity in Almond (Prunus dulcis). Ann. Bot. 97, 57–63. https://doi.org/10.1093/aob/mcj013.

Zamir, D., Tanksley, S.D., Jones, R.A., 1982. Haploid selection for low temperature tolerance of tomato pollen. Genetics 101, 129 LP–137.

Zhang, L., Ferguson, L., Whiting, M.D., 2018. Temperature effects on pistil viability and fruit set in sweet cherry. Sci. Hortic. (Amsterdam) 241, 8–17. https://doi.org/10.1016/J.SCIENTA.2018.06.039.

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D.B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.-L., Elliott, J., Ewert, F., Janssens, I.A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., Peng, S., Peñuelas, J., Ruane, A.C., Wallach, D., Wang, T., Wu, D., Liu, Z., Zhu, Y., Zhu, Z., Asseng, S., 2017. Temperature increase reduces global yields of major crops in four independent estimates. Proc. Natl. Acad. Sci. U. S. A. 114, 9326–9331. https://doi.org/10.1073/pnas.1701762114.

Zinn, K.E., Tunc-Ozdemir, M., Harper, J.F., 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. J. Exp. Bot. 61, 1959–1968. https://doi.org/10.1093/jxb/erq053.